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Sitosterolemia – 10 years observation in two sisters

Lara Veit[1], Gabriella Allegri Machado[1], Céline Bürer[1], Oliver Speer[2], Johannes Häberle[1]

[1] Division of Metabolism and Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland

[2] Division of Haematology and Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland & Spital Thurgau AG, Institut für Labormedizin, Frauenfeld, Switzerland

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Abstract

Familial hypercholesterolemia due to heterozygous LDL-receptor mutations is a common inborn errors of metabolism. Secondary hypercholesterolemia due to a defect in phytosterol metabolism is far less common and may escape diagnosis during the work-up of patients with dyslipidemias. Here we report on two sisters with the rare, autosomal recessive condition, sitosterolemia. This disease is caused by mutations in a defective ATP-binding cassette sterol excretion transporter, leading to highly elevated plant sterol concentrations in tissues and to a wide range of symptoms. After a delayed diagnosis, treatment with a diet low in plant lipids plus ezetimibe to block the absorption of sterols corrected most of the clinical and biochemical signs of the disease. We followed the two patients for over ten years and report their initial presentation and long-term response to treatment.

Take-home message

Elevated cholesterol concentrations are not always identical with familial hypercholesterolemia, and sitosterolemia should be included as a treatable differential diagnosis. This is especially relevant if family screening does not confirm familial hypercholesterolemia in at least one of the parents.

Key words: sitosterolemia, familial hypercholesterolemia, xanthoma, phytosterols, *ABCG5* or the *ABCG8* gene

Compliance with Ethics Guidelines

Lara Veit, Gabriella Allegri Machado, Céline Bürer, Oliver Speer and Johannes Häberle declare that they have no conflict of interest.

This article does not contain any studies with human subjects performed by the authors. The family agreed to publication of this report.

Authors' contribution

LV and JH have planned the conception and design of the study. LV, GA and CB have collected patients' data. OS has performed laboratory investigations. LV and JH have drafted the manuscript and designed the figures, which were revised by all authors.

Introduction

Sitosterolemia (MIM #210250) is a rare, autosomal-recessive disease characterized by accumulation of plant sterols in blood and tissues (Patel and Salen 2010). Sitosterolemia was first described in 1973, when elevated plasma levels of plant sterols (sitosterol, campesterol and stigmasterol) were detected in two sisters of European descent who had extensive tendon xanthomas but normal plasma cholesterol levels (Bhattacharyya and Connor 1974). Although the exact prevalence of sitosterolemia is not known, it may be underdiagnosed and in fact more common than the estimated incidence of 1 in 1,000,000 (Kidambi and Patel 2008; Park et al. 2014; Yoo 2016). In fact, recent data indicate a prevalence of the disease more than 1 in ~200,000 individuals among the general population (Tada et al. 2018a).

Sitosterolemia is caused by mutations in either the *ABCG5* or the *ABCG8* gene (Berge et al. 2000; Lee et al. 2001; Lu et al. 2001), two oppositely oriented genes located on chromosome 2p21 (Patel et al. 1998). Inactivating mutations of both alleles at either the *ABCG5* or *ABCG8* locus cause sitosterolemia. A single report of a patient with sitosterolemia who is heterozygous for a mutation in both genes has been reported (Tada et al. 2018b). While almost all Asian patients carry *ABCG5* mutations, Caucasian patients more often present *ABCG8* mutations (Park et al. 2014; Patel and Salen 2010; Tada et al. 2015; Wang et al. 2004). *ABCG5* and *ABCG8* are coding for sterolin 1 and sterolin 2, respectively, which form an obligate heterodimer and ATP-binding cassette transporter (Fig. 1) (Berge et al. 2000; Lee et al. 2001; Lu et al. 2001; Wang et al. 2004). Loss of function of this transporter leads to increased absorption of all dietary sterols and thus to progressive accumulation of sterols. While plasma cholesterol is often normal in adult patients with sitosterolemia, pediatric patients can show severe hypercholesterolemia (Patel and Salen 2010; Yoo 2016).

Clinically, sitosterolemia is very heterogeneous with a spectrum that extends from the patients being asymptomatic to early lethal cases (Wang et al. 2004; Yoo 2016). Typical manifestations

are listed in Table 1. The complete clinical expression of sitosterolemia may not be known due to under-diagnosis (Merkens et al. 1993).

To diagnose this disorder requires the measurement of plant sterol concentrations in plasma using gas chromatography-mass spectrometry (GC-MS) or high pressure liquid chromatography (HPLC). Standard enzymatic cholesterol analysis does not distinguish between plant sterols and cholesterol and therefore can lead to false high total cholesterol concentrations (Patel and Salen 2010; Yoo 2016). Mutation analysis can be made by sequencing the *ABCG5* and *ABCG8* gene (Niu et al. 2010; Yoo 2016).

Treatment of sitosterolemia aims to lower plasma plant sterols and, if elevated, cholesterol concentrations and to prevent complications (Merkens et al. 1993; Yoo 2016). To achieve this, a combination therapy is usually needed (Yoo 2016): dietary restriction of both animal- and plant-based sterols (Belamarich et al. 1990; Merkens et al. 1993; Parsons et al. 1995); bile acid sequestrants, e.g. cholestyramine (Belamarich et al. 1990; Merkens et al. 1993; Parsons et al. 1995); and ezetimibe to reduce sterol uptake (Lutjohann et al. 2008; Merkens et al. 1993; Othman et al. 2015; Salen et al. 2006; Salen et al. 2004). Treatment results in a reduction in plasma concentrations of cholesterol and plant sterols and regression of existing xanthomas (Merkens et al. 1993; Yoo 2016). Thus, sitosterolemia is a treatable condition, especially when diagnosed and treated early, which underlines the importance of correct diagnosis and management of sitosterolemia (Yoo 2016).

Here we report on two patients with sitosterolemia who were initially not recognized to have the disorder, who now have been treated for more than 10 years.

Case Reports

Patient 1, first child of non-consanguineous Bosnian parents, was referred to our unit at age 8.5 years after her parents gave a one year history of her developing bluish soft swellings on the extensor surfaces of both her knees and elbows. On physical examination, she was found to also have similar lesions on the buttocks and the Achilles tendons. Previously, one of the lesions had been biopsied and the diagnosis of "xanthoma disseminatum" was made. Her fasting blood total and LDL-cholesterol levels were 12.1 mmol/L and 10.2 mmol/L, respectively. Treatment with statins did not significantly lower her LDL-cholesterol and the patient proceeded to develop xanthelasmas. Both of her patents had cholesterol levels that were only slightly elevated above the upper limit of normal, already raising suspicion about the possibility of a LDL-receptor defect. Her younger sister was also tested and had a total cholesterol of 7.3 mmol/L and LDL-cholesterol of 5.2 mmol/L. The family history beyond that was uneventful.

Sequencing of the coding exons of the genes encoding for the LDL-receptor and apolipoprotein B in all family members did not reveal mutations. Finally, after 2.5 years, the plasma phytosterols were measured by gas chromatography-mass spectrometry (GC-MS) and found to be very elevated (sitosterol 555 µmol/L, ref. 0.6 - 14.88; campesterol 353 µmol/L, ref. 0.52 - 17.65) (Fig. 2). Genomic DNA from peripheral white blood cells was used for sequencing all exons of *ABCG5* and *ABCG8* (performed at University of Texas Southwestern Medical Center) (Hubacek et al. 2001). This revealed that both patients were homozygous for a stop mutation at position 446 of *ABCG5* (p.Arg446*) and that both parents were carriers of the nonsense mutation.

When diet therapy for about 6 months plus colestid treatment for about 2 months did not lead to a reduction in plasma sterol levels, ezetimibe was added to the medical regimen. Treatment with ezetimibe led to a significant decrease of total and LDL-cholesterol levels, as well as to a decrease in concentrations of phytosterols (Fig. 2). In patient 1, treatment with ezetimibe lead

to an initial normalization of total and LDL-cholesterol but levels increased later slightly above the reference ranges, while in patient 2 the concentrations of total and LDL-cholesterol remained normalized until the end of the observation period (Table 2 and Fig. 2). During treatment, xanthoma in patient 1 disappeared after several years with minimal bluish skin discoloration remaining in the respective areas of knees and elbows.

To investigate for known complications of sitosterolemia, platelet aggregation tests, thrombo-FACS, platelet electron microscopy, ferritin, free hemoglobin (Hb), and Hb-carbonmonoxide (CO) were analysed and found normal in both patients. Specifically, there was no evidence for the presence of hemolysis, abnormally shaped erythrocytes (stomatocytes) or thrombocytes (macrothrombocytopenia). Platelet aggregation was normal, as was expression of platelet surface proteins GPIb, IIb, and IIIa. All examined markers could be stimulated by thrombin.

In patient 1, echocardiography at 12.8 years of age showed hyperechogenic and thickened areas at the base of the mitral and tricuspid valves. One year later, a calcification had developed on the sinotubular junction, but without causing stenosis. These findings remained unchanged until the end of the observation period. When ECG showed signs of a disturbed repolarization, a CT angiography was done to exclude coronary artery involvement. This revealed normal coronary arteries but calcified plaques in the left ventricular outflow tract and in the wall of the ascending aorta (Fig. 3). Patient 2 showed no cardiac involvement but additional morbidities including infantile epilepsy and mixed hyperandrogenemia (acne, mild hirsutism and oligomenorrhoea).

Parents of the reported patients provided their consent to publication of this report.

Discussion

Sitosterolemia is a rare and probably underdiagnosed autosomal recessive disorder of lipid metabolism. The clinical presentation is variable, making early diagnosis challenging. Since the

condition can lead to development of premature coronary atherosclerosis, which can be prevented by treatment, it is important that the disease be diagnosed early. Here we review the clinical presentation and successful management of two sisters with the disorder. In the index patient, the diagnosis of the disease was delayed by more than two years. After consultation with several specialities, the patient was assumed to have familial hypercholesterolemia (FH) based on an elevated LDL-cholesterol levels. However, treatment with statins alone failed, which is typical for sitosterolemia patients and a useful clue to the diagnosis. The initial misdiagnosis is likely the result of sitosterolemia being a rare disease, with less than 100 cases described to date (Bazerbachi et al. 2017). Besides several case reports, the largest case series reports 13 patients from 8 independent families, who were all treated at a single institution (Wang et al. 2014).

Diagnosis of sitosterolemia requires a high level of awareness, since the standard lipid profiles provide no specific hints. Rather, specific phytosterol analysis must be requested (Bazerbachi et al. 2017). Another clue is that parents are normocholesterolemic, as it was in principle the case in the reported family with only slight cholesterol elevations in the parents. In case of absent hypercholesterolemia in the parents of a patient, and if xanthoma are present in typical body areas, physicians should include sitosterolemia in the differential diagnosis of elevated cholesterol concentrations. Confirmation of the disease may include analysis of *ABCG5* and *ABCG8* genes but mutation analysis is not required to make the diagnosis. Both our patients were homozygous for a previously reported stop mutation (c.1336C>T, p.Arg446*) in the *ABCG5* gene (Mannucci et al. 2007). This mutation was found in at least 19 patients from different origins: Iranian (Mannucci et al. 2007), Japanese (Tada et al. 2015; Togo et al. 2009), Chinese (Niu et al. 2010), Romanian (Rios et al. 2010), Korean (Park et al. 2014), Turkish (Li et al. 2016) and Pakistani (Bazerbachi et al. 2017), plus further patients (Fang et al. 2018; Kratz et al. 2007; Wang et al. 2014). Our patients are of Bosnian origin and therefore serve as

examples for genetic heterogeneity at both loci. Previously, Asian patients more often carried *ABCG5* mutations, while Caucasian patients were more frequently affected by mutations in *ABCG8* (Berge et al. 2000; Kidambi and Patel 2008; Lu et al. 2001; Patel and Salen 2010).

The importance of early diagnosis and initiation of treatment is underlined by the different clinical and biochemical course in our patients: while patient 1 presented with multiple clinical signs including cardiac calcifications, patient 2 was asymptomatic. Also, maximum concentrations of cholesterol and of phytosterols in patient 1 were noticeably higher than in patient 2. While there are no apparent differences between patients with mutations in *ABCG5* or *ABCG8* (Lee et al. 2001; Lu et al. 2001), there is considerable heterogeneity in the clinical and biochemical phenotypes of patients even with identical genotypes (Hu et al. 2014; Mannucci et al. 2007; Park et al. 2014; Wang et al. 2004). The cause of this variability is not known, but time of diagnosis and start of treatment may contribute. Additional factors may include *NPC1L1* polymorphisms leading to reduced sterol absorption and LDL-cholesterol levels (Cohen et al. 2006; Hu et al. 2014; Kidambi and Patel 2008; Tang et al. 2009; Teslovich et al. 2010), variations in other lipid-related genes influencing cholesterol absorption or other pathways (Teslovich et al. 2010), and differences in dietary sterol intake (Hu et al. 2014), the latter especially in infants consuming different diets (Merkens et al. 1993).

Management of our patients included cardiac investigations, regular monitoring of lipid and sterol profiles, and liver transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) to rule out liver involvement (Bazerbachi et al. 2017). Hematological investigations showed no stomatocytes, normal thrombocyte morphology and function, and no disturbance of hemostasis (Niu et al. 2010; Patel and Salen 2010; Wang et al. 2011; Wang et al. 2014; Yoo 2016). Recently, it was suggested that “elevation of LDL-cholesterol seems to be the major cause of development of atherosclerosis and not the elevation of sitosterols” (Tada et al. 2018a)

rendering normalization of the cholesterol concentrations, as achieved in both patients, the primary treatment target.

Infantile epilepsy, present in patient 2, has been reported before in a single Chinese sitosterolemia patient (Hu et al. 2014), but a causal relationship is unclear. As well, sitosterolemia may have contributed to altered androgens and mixed hyperandrogenemia since plant sterols can serve as precursors for steroid hormones (Malaviya and Gomes 2008). However, the effect of phytosterols on the endocrine system is not completely understood. It is known that absorbed plant sterols mainly accumulate in the liver, gonads and adrenal glands indicating a high affinity to steroid-synthesizing tissues (Moghadasian 2000). Further supporting a role of plant sterols, phytosterol-enriched margarines led to a slight increase in testosterone in 10 women (Ros et al. 2007).

In summary, elevated cholesterol concentrations are not always identical with FH, and sitosterolemia should be included as a treatable differential diagnosis. This is especially relevant if family screening does not confirm FH in at least one of the parents.

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Legends to tables

Table 1: Possible clinical presentations of sitosterolemia

Table 2: Basic clinical and biochemical data of study patients

Legends to figures

Legend Figure 1: Model for absorption and secretion of cholesterol and plant sterols: Physiologically, the ABCG5/G8 transporter pumps absorbed nutrition sterols (absorbed through the Niemann-Pick-C1-like 1, short NPC1L1) back into the intestinal lumen or into the bile, with a preference for non-cholesterol sterols, if they are present. It occurs in the apical membrane of small intestine enterocytes and hepatocytes.
BA: bile acids, PL: phospholipids, BSEP: bile salt export pump, BDR3: multiple drug resistance protein 3, NTCP: sodium/taurocholate co-transporter.

Legend Figure 2: Lipid levels, symptoms and treatments of both patients during the observation period

Legend Figure 3: Clinical pictures and CT scan of patient 1. Xanthelasma at age 11.2 (A), 11.9 (B) and 13.3 years (C). Xanthomata at age 8.8 (D), 11.2 (E) and 13.3 years (F). CT scan with cardiac calcifications at age 13.5, arrows indicate site of calcifications (G, H).